

Welfare and management practices of free-ranging yaks (*Bos grunniens*) in Bhutan

N Dorji^{*†}, M Derks[‡], PWG Groot Koerkamp[‡] and EAM Bokkers[§]

[†] Department of Animal Science, College of Natural Resources, Royal University of Bhutan, Lobesa, Bhutan

[‡] Farm Technology Group, Wageningen University & Research, PO Box 16, 6700 AA, Wageningen, The Netherlands

[§] Animal Production Systems Group, Wageningen University & Research, PO Box 338, 6700 AH, Wageningen, The Netherlands

* Contact for correspondence: nepdorjion186@yahoo.com/nedup@cnr.edu.bt

Abstract

Inaccessibility of veterinary and livestock extension services, and shortages of labour and forage could potentially impact the welfare of yaks (*Bos grunniens*) in Bhutan. The objective of this study was to assess practices relating to the welfare and management of free-ranging yaks in Bhutan and explore variations between different yak-farming regions. We interviewed herders and observed the behaviour and health status of their animals, using an adaptation of the Welfare Quality® protocol, in three yak-farming regions (east, central and west) of Bhutan between October 2018 and January 2019. In total, for 567 cows and 549 calves, integumentary condition, body cleanliness, ocular and nasal discharge, diarrhoea, signs of damage, and gait were scored. In addition, we assessed 324 cows and 272 calves for avoidance distance and examined 324 cows for subclinical mastitis. The behaviour of the herds was observed in six consecutive 20-min blocks with each block divided into two stages. The first stage (5 min) consisted of counting the number of animals eating, lying down, standing idle and walking. The second stage (15 min) consisted of counting the number of events of agonistic, allogrooming, flehming, self-licking, rubbing/scratching and playing behaviour. Avoidance distance differed between regions for calves, but not for lactating cows. Integumentary lesions, dirty body areas, nasal discharge, ocular discharge, signs of diarrhoea, subclinical mastitis and lameness were virtually absent. A few instances of agonistic behaviour (6% of all counted behavioural events) and flehming behaviour (5% of all counted behavioural events) were observed. Yaks in the central and western regions exhibited more scratching and rubbing behaviour than those in the eastern region. Herders perform a variety of painful management practices (castration, ear tagging, nasal septum piercing) without analgesia, which is a prominent welfare issue. Furthermore, mortality among yaks is relatively high and water sources often dirty, creating a health risk. Nevertheless, the welfare status of yaks living in various regions of Bhutan was assessed as good at the time of visit.

Keywords: animal health, animal welfare, behaviour, livelihood, pastoralists, yak

Introduction

Highland pastoralists in Bhutan depend primarily on yak (*Bos grunniens*) farming for their livelihoods. These yak-based transhumant communities migrate with their herds in the Himalayas between summer (5,000 m above sea level) and winter (2,500 m above sea level) in response to forage availability and extreme climatic conditions. Yaks are kept for a variety of purposes, including milk and hair. In some areas they are also used as pack animals for transportation and traction. The primary constraints in transhumant yak-farming systems are forage shortages in the winter, labour shortages and the inaccessibility of veterinary and livestock extension services (Derville & Bonnemaire 2010; Wangchuk & Wangdi 2015; Dorji *et al* 2020).

Although yaks are well adapted to their environment both anatomically (eg large chest and heart capacity and a large lung-surface area relative to body size; Wiener *et al* 2003)

and physiologically (eg low haemoglobin count, but high affinity for the presence of low atmospheric oxygen at higher altitudes; Wiener *et al* 2003), their performance can be affected by harsh conditions in the mountains, combined with feed shortages in the winter and spring (Gyamtscho 2000). During periods of feed shortage, herders try to provide supplements (eg maize and wheat flour) and cattle concentrates (Wangchuk & Wangdi 2015), but their financial resources tend to be limited. Furthermore, yak herders have scant access to veterinary services, as the nearest livestock extension centres are generally at walking distances of at least 1–3 days (winter) and up to ten days (summer) from their herds (Derville & Bonnemaire 2010). For this reason, yak herders try to treat diseases and injuries by applying indigenous knowledge and ethno-veterinary medicines (Dhendup 2015). ‘Gid’ (*Coenurus multiceps multiceps*), is a typical disease of central and western Bhutan which is

caused by an endoparasite that forms cysts in the animal's brain and poses a major risk to health, with increased mortality rates among young yaks (Gyamtscho 2000).

In addition to food shortages and health issues, the skills and knowledge of herders concerning the handling and restraint of the animals are essential factors that are likely to affect the welfare of the animals (Waiblinger *et al* 2006). Yaks are kept on rangelands without fences and as they have substantial freedom to move, gathering, restraint and handling for milking, vaccination or shearing can cause them stress.

The development philosophy of Bhutan is based on the quality of life for Bhutanese people, rather than on the economy, and is expressed in terms of 'Gross National Happiness.' As sentient beings, yaks are included in this philosophy, and this includes optimal health and welfare. To date, however, animal-protection regulations are still at the developmental stage and the assessment of animal welfare has yet to be properly implemented. As a result, no information on the welfare status of kept animals is available. Despite yak welfare not perhaps being at the forefront of yak herders' minds, insight into the welfare status and underlying issues that impact the welfare of their animals would be of benefit to yak-based communities. For example, improvements in animal welfare could help reduce mortality and morbidity, thereby enhancing product quality and yielding financial benefits for farmers (Appleby & Mitchell 2018). So far, to the authors' knowledge, there have been no studies assessing the welfare of yaks in free-ranging systems. Studying the welfare of free-ranging yaks is challenging, both geographically (animals are reared under harsh environmental conditions in very remote mountainous areas) and contextually (animals are not used to being handled and mainly roam free).

In light of the issues outlined above, the objective of this study was to assess practices relating to the welfare and management of free-ranging yaks in Bhutan and to explore variations between different yak-farming regions (east, central and west), in order to gain an insight into the welfare status of yaks in Bhutan. We hypothesised that the health and welfare condition of yaks was likely to be impaired due to feed shortages, inaccessibility of veterinary and livestock extension services and the application of traditional management practices (Dorji *et al* 2020). Given the variations likely to exist between different regions in terms of herders' opinions, traditions and beliefs as regards yak management, we also hypothesised that welfare indicators might yield different outcomes for different yak-farming regions.

Materials and methods

Ethical approval

This study (Application ID: 5069623915B73E79DE0037) was approved by the research ethics committee of UWICER-DoFPS in Bhutan. Approval was required for conducting the interviews and visiting the protected areas in which yak herders are located. During the field visit, the yak herders granted oral consent to participate in the study.

Herd selection and visits

Bhutan has ten districts, encompassing a total of 25 blocks (a local administrative unit comprising several villages) in which yak farming operates (DoL 2018). The yak-farming districts can be stratified into three regions, which are characterised by differences in culture and tradition, yak products, breeding practices and sources of livelihood (Derville & Bonnemaire 2010). Within each of these regions, one block was selected. Within the blocks that were selected — Merag (east), Saephu (central) and Laya (west) (Figure 1) — 59 yak herds (east, 19; central, 20; west, 20) from 13 villages (east, 3; central, 6; west, 4) were visited while on their winter rangelands, located at altitudes of around 3,000 m above sea level. Herds were selected using a sampling method based on the number of herds in the areas and their distance from the yak-farming villages (the investigator considered visiting herds that were at a walking distance of no more than one day from the village) (Dorji *et al* 2020). Herds were visited between October 2018 and January 2019 as this was when herds remained in one location for the longest and winter rangelands are easier to access than summer rangelands, which are at higher altitudes and even more remote. The protocol and questionnaire were pre-tested with one yak herd and revised accordingly. Face-to-face interviews with yak herders and herd assessments were first conducted in the eastern region (October), followed by the western (November/December) and central regions (December/January). The resulting time effect in the data collection was unavoidable, due to the extensive distances between regions.

Interviews with herders

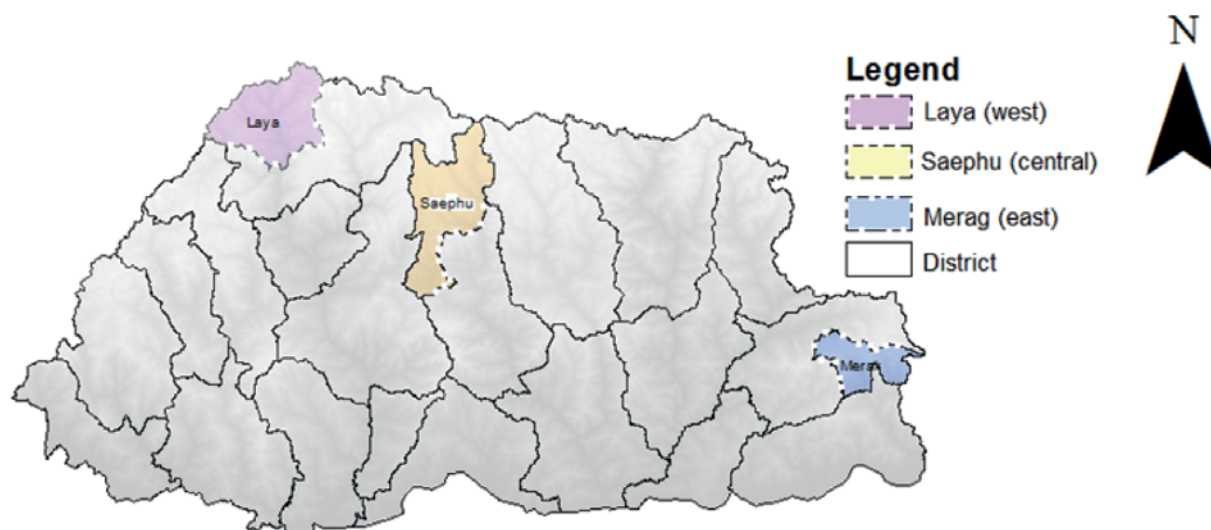
Face-to-face interviews were conducted with yak herders, using a semi-structured questionnaire with both open- and close-ended questions. The questionnaire was divided into three sections:

- Basic respondent information, including age, sex, level of education and years of yak herding;
- Current yak management and husbandry practices relating to milk yields per cow, assistance during calving, mortality and cause, access to veterinary and livestock extension services, painful practices (disbudding, de-horning, nose-ringing, male castration) and the use of anaesthesia or analgesics;
- Opinion of herders with regard to naming yaks, and whether herders think that yaks have feelings (ie are sentient beings).

The complete questionnaire is included online in Supplementary material (Table S1).

Herders were interviewed for approximately 30 min in the Bhutanese national language (western and central region) or in the local dialect, *sharchopkha* (eastern region). The questionnaire was translated from English into the national language and verified by a qualified translator. As a native speaker of the local dialect in the eastern region, the interviewer (ND) was able to translate the questions directly during the interview.

Figure 1



Locations of the three study areas in Bhutan.

Welfare assessment

The applied welfare protocol and assessment procedure was based on elements of the Welfare Quality® protocol for cattle (Welfare Quality® 2009) and included specific features of yaks, drawing on literature (eg excitability: Wiener *et al* 2003; eg idling and grazing behaviour: Liu *et al* 2019) and expert knowledge on yak biology and behaviour. This information made it possible to conduct the assessment in the open field under mountainous conditions. The yak-welfare assessment protocol involved 3 h of animal-based measurements.

Yaks were observed at two different times during the day: lactating cows with their calves being restrained during milking, and the whole herd when free-ranging on the rangeland.

Assessment during the milking procedure

During the milking procedure, cows and their calves had their response to an unfamiliar human assessed, as well as integumentary condition, body cleanliness, ocular and nasal discharge, and signs of diarrhoea and damage (eg ear tags, nose rings). They were also scored for subclinical mastitis and gait. The procedures deployed are described below.

Avoidance distance

From a total of 50 herds (east, 14; central, 17; west, 19), 324 lactating cows (east, 115; central, 62; west, 147) and 272 calves (east, 92; central, 80; west, 100) were assessed for avoidance distance, without disturbing the daily milking routine. It was performed on calves before milking, and on lactating cows after milking (des Roches *et al* 2016). The unfamiliar observer approached the animal from a distance of 3 m. After the animal became aware of the observer's presence, the observer lifted one arm at an inclination of approximately 45° from the body and walked towards the animal at a rate of one step per second. When the animal

responded by moving away (eg stepping backwards, moving or shaking its head, or stepping forwards), the distance (in cm) between the observer's hand and the animal at the time of withdrawal was estimated visually. Given that yaks are easily agitated, it was not possible to conduct exact distance measurements using a measuring tape. If the observer was able to touch the animal, the distance was scored as 0 cm. In some herds, the avoidance-distance test could not be conducted according to the procedure described, as the cows were not milked during the visit (east, 1 herd; central, 1 herd) or because the herder did not grant permission (east, 4 herds; central, 2 herds; west, 1 herd).

Health examination

From a total of 57 herds (east, 18; central, 19; west, 20), 567 lactating cows (east, 267; central, 124; west, 176) and 549 calves (east, 257; central, 117; west, 175) were inspected to assess integumentary condition, body cleanliness, ocular and nasal discharge, and signs of diarrhoea (Table 2). Each of these health indicators was scored as either present or absent. An equally proportioned body side of each animal (left or right) was scored for integumentary condition and body cleanliness. If the right side of the first animal was selected for inspection, the left side of the next animal was inspected, and so on. For safety reasons, observations were performed from a distance of 3–6 m, given that yaks are easily agitated. Observations around milking time could not be performed in one eastern and one central herd, as the cows were not milked.

Invasive procedures

From a total of 57 herds (east, 18; central, 19; west, 20), 567 lactating cows (east, 267; central, 124; west, 176) and 549 calves (east, 257; central, 117; west, 175) were inspected for damages inflicted by the herders (eg nose rings and ear tags).

Table 2 Summary of the yak health indicators adapted from Welfare Quality® (2009).

Variable	Description
Integument condition	Animals were inspected for swellings, fresh wounds, or lesions in the body region (between neck and rump region), front limb (including knee), and hind limb (including the hock). These were recorded separately. Lesions and wounds were counted if the lesion or wound was at least ~2.5 cm in diameter
Body cleanliness	Animals were examined for cleanliness in the body area and hind limb. The animal was scored as clean (less than 25% of the area in question covered with plaques, or less than 50% of the area covered with liquid dirt) or dirty (25% of the area in question or more covered with plaques, or more than 50% of the area covered with liquid dirt)
Ocular discharge	Presence of any visible flow (wet or dry) from the eyes of at least 3 cm in length
Nasal discharge	Presence of any visible flow (transparent, coloured) from the nostril
Diarrhoea sign	Animals were considered to suffer from diarrhoea if the tail was soiled with at least a hand-sized section of loose, watery manure
Hip bone	Depression between the hip bones was defined as a visible hip bone (Edmonson et al 1989)

Subclinical mastitis

In all, 56 herds (east, 17; central, 19; west, 20) were tested for subclinical mastitis using the California Mastitis Test (sodium lauryl sulphate and 1:10,000 bromocresol purple) (Sargeant et al 2001). About 50% of the lactating yak cows in a herd (east, 131; central, 71; west, 106) were randomly selected and tested. The herder was provided with a test paddle and asked to fill it with two to three squirts of milk from each quarter in a designated cup. An equal amount of reagent was added to each separate cup containing milk. The mixture was swirled gently for 5 s, and the reaction scored as either negative (no thickening of the mixture) or positive (gel-like appearance of the mixture) for subclinical mastitis. The paddle was rinsed with disinfectant after each test.

Gait score

From a total of 57 herds (east, 18; central, 19; west, 20), 567 lactating cows (east, 267; central, 124; west, 176), 549 calves (east, 257; central, 117; west, 175) and six bulls (west) were scored for gait condition at two occasions: (i) in the morning, when the animals were gathered for milking; and (ii) after milking, when the herder drove the herd to the rangeland. The observer maintained a distance of around 10 m from the animals, observing them as they were gathered for milking and/or driven to the rangeland. A two-point scale (not lame or lame) was used to classify the gait of the yaks and a yak was scored as lame if it was clearly limping while walking. This is comparable to a score of ≥ 3.0 based on the protocol developed by Flower and Weary (2006).

Animal observations when on the rangeland

Behavioural observations when the animals were on the rangeland included all animals (cows, bulls, heifers, calves) that were present. At some locations, several herds were using a common rangeland (east, 4; central, 12; west, 10) for grazing. In these cases, the herds were observed and assessed together. As a result, a total of 35 herds (east, 15; central, 8; west, 12) were assessed for behaviour. Binoculars were used for observations. The number of animals prior to and after completion of behavioural observations was counted.

Animals were scored for behavioural states (5 min), followed by behavioural events (15 min). Observations were repeated six times (6×20 min) and were concluded with an additional 5-min period of behavioural-state observation. Behavioural observations were performed either in the morning or afternoon, depending on the local situation. The behavioural states were assessed by counting the number of animals eating, lying down, standing idle and walking once within the 5-min period (Table 3). All instances of agonistic behaviour (head butting, chasing, fighting), allogrooming (adult-adult licking, adult-young licking), sexual behaviour, self-licking, rubbing/scratching, and playing behaviour were counted within the 15 min immediately following the observation of behavioural states (Table 3).

Water source inspection

The total number of sources of drinking water available to the yaks was recorded, as well as whether the source was a running or stagnant one. A sample was taken from each water source in a transparent plastic cup and held up to the light to assess water cleanliness. Water was classified as either clean (clear, with no evidence of crusts of dirt or decay), partly dirty (clear, but containing particles) or dirty (coloured, eg brown, green or red).

Data processing and analysis

All data were entered into an Excel® spreadsheet for each yak herd and exported to R (version 3.5.0) for analysis (R Team Core 2018). Data were tested for normality using the Shapiro-Wilk test, and both parametric and non-parametric tests were performed accordingly. Regional differences (east for Merag, central for Saephu and west for Laya) in respondents' age, herding experience and the size of yak herds were compared based on the median and using the Kruskal-Wallis test. The median was used rather than the mean, as the variables were skewed (eg in terms of respondent age). The effect size was estimated using the epsilon-squared (ϵ^2) method to assess the strength of relationships between variables. Multiple responses for the causes of yak mortality were recoded dichotomously as responses 'yes' or

Table 3 Ethogram of yak behaviour (adapted from Welfare Quality® 2009).

Variable	Description
<i>Behavioural state</i>	
Eating	Taking grass or forbs, woody twigs and leaves from trees and shrubs into the mouth
Lying down	Lying on the chest on the ground
Standing idle	Standing posture without performing any other activity
Walking	Walking without eating
Others	All other behaviours not included in the lists (eg suckling, licking)
<i>Behavioural event*</i>	
Head-butting	Interaction with physical contact (butting, hitting, thrusting, striking or pushing). One yak butting or pushing another by the forehead or horns; the receiver may or may not give up its position (but does not flee)
Chasing	One yak makes another yak flee (with or without physical contact)
Fighting	Two yaks push their foreheads, horn bases or horns against each other while planting their feet on the ground, with both exerting force against each other
Allogrooming	Touch body parts of group-mate with the tongue, but not licking around the anal region or prepuce
Self-licking	Touch own body parts with tongue
Rubbing and scratching	Use of horn or legs to scratch own body part or rub any part of the body against any object (eg soil, rock, pole)
Flehming behaviour	Bull rests chin on yak cow and exhibits flehming behaviour (sniffing female genitalia, followed by raising the nose into the air with the mouth slightly opened)
Play behaviour	Fast galloping with the tail lifted up, interrupted by sudden change of direction, hind-leg kicking, body rotation and twists

* A new bout starts if the same animals restart the specific type of behaviour after more than 10 s.

'no.' The percentages for the causes of yak mortality, the frequency of livestock services received, the frequency of deworming and ectoparasite treatment, and painful practices were calculated.

The percentages of calves and cows per herd with integumentary alterations, body cleanliness, ocular and nasal discharge and signs of diarrhoea, lameness, and ear tags were calculated. Left- and right-side animal-body scores for integumentary alterations and body cleanliness were summarised into a single variable, as integumentary alterations and body uncleanliness were virtually absent. A score of 'present' was given if an animal displayed evidence of either integumentary alteration or body uncleanliness. In addition, the percentage of cows presenting with a positive outcome on the clinical mastitis test for at least one-quarter was also calculated.

Data on avoidance distance and behaviour were analysed using the following packages in R: car (Fox & Weisberg 2019), MASS (Ripley *et al* 2013), lme4, glmer (Bates *et al* 2015) and lsmeans (Lenth 2016). A mixed model was used with the herd treated as a random effect because of expected herd size variability within the regions. In all models, region was treated as a fixed effect. In the preliminary analyses,

differences in the avoidance distance of calves and cows relative to an unknown person was fitted using a linear mixed model (LMM). Comparisons between the residuals and the fitted values, and between the residual quantile and the quantile plot of the model revealed deviations from normality (Bolker *et al* 2009). According to the Shapiro-Wilk test, the residuals of the model did not meet the assumption of normality. For this reason, a constant value of 1 was added to the original avoidance-distance data, after which the data were log-transformed and used in a linear mixed model. In addition, a generalised linear mixed model (GLMM) using a binomial logit-link was used to determine whether animals could or could not be touched in the avoidance-distance test. If the fixed effect was significant ($P < 0.05$), the Corrected Bonferroni method of Dunn's test was used for *post hoc* pair-wise comparisons (Dinno 2017). The model results are provided in Supplementary material (Tables S4 and S5).

The starting and ending times of the observations on the rangeland differed, due to weather, herder activity (eg salt feeding, duration of milking procedure) and the visibility of the animals in the landscape. Behavioural data were therefore classified as morning (all observations between

0625 and 1200h) and afternoon (all observations between 1500 and 1750h), and each group was analysed separately. In the afternoon observation, only one herd from the central region was observed for the behaviour, and was excluded from further analysis. For every 5 min of behavioural observation, the proportion of each behaviour was calculated as the number of individual animals performing the behaviour divided by the total number of animals observed. The mean for each behavioural state was calculated from the seven observation periods. The Corrected Bonferroni method of Dunn's test (Dinno 2017) was used for *post hoc* pair-wise comparisons when the fixed effect was significant ($P < 0.05$). Similarly, the number of behavioural events per animal per herd was calculated as the count of each event happening divided by the total number of animals. The mean for each behavioural event was calculated from the six observation periods. The number of events per animal per 15-min period is reported, as the behaviour of the yaks was observed for 15 min, and the number of yaks observed during that period could vary. The mean for each behavioural type per animal per 15 min was calculated.

Results

Respondent characteristics

Of the 59 yak herders approached, 52 (88%) agreed to participate in the interview. The others responded that they did not have time for the interview. Most of the eastern region responders were male (81%), whereas the central (73%) and western (82%) respondents were mostly female. Most were illiterate (east, 94%; central, 84%; west, 82%), and the rest had attended primary education. The median age of the respondents varied by region (east, 55 years; central, 38 years; west, 41 years) ($P < 0.05$; $\xi^2 = 0.13$). There were no regional differences in herding experience (east, 31.5 years; central, 20.0 years; west, 23.0 years; $P > 0.05$; $\xi^2 = 0.04$). Although the median herd size did not differ by region (east, 52; central, 49; west, 50; $P > 0.05$; $\xi^2 = 0.07$), the median number of calves (east, 12; central, 4; west, 8; $P < 0.05$; $\xi^2 = 0.26$) and lactating cows did differ significantly (east, 15; central, 6; west, 8; $P < 0.05$; $\xi^2 = 0.27$). The observed proportions were 19% cows (east, 27%; central, 16%; west, 14%) and 18% calves (east, 26%; central, 16%; west, 13%). The rest of the herd consisted of bulls, heifers and dry cows.

General management practices

All of the herds were reared using a free-range grazing system, and the common general floral species composition included sedge (*Carex* spp), grass (*Bromus* spp, *Agrostis* spp, *Festuca* spp, *Poa* spp), forbes (*Anaphalis* spp, *Bistorta* spp, *Cyananthus* spp, *Primula* spp) and shrubs (*Rhododendron* spp, *Juniperus* spp). None of the herders drove animals to water sources. In all of the herds, supplements (eg maize flour mixed with common salt) were provided to calves and to weak and lactating animals during milking in the winter and spring. Young stock, cows and bulls had access to rangeland 24 h a day. The majority of herds (east, 88%; west, 75%) had access to sources of

visibly clean-running drinking water, while the rest had at least one source of natural running water that was qualified as partly dirty (east, 1–3 per herd; west, 1–2 per herd). In the central region, most herds (89%) had at least one source of running drinking water that was qualified as dirty. In addition, in the central region, one-third of the yak herds visited also had an artificial water pond (stagnant). All of these ponds were rated as either partly dirty or dirty.

In the central and western regions, a small, simple night shelter was provided close to the campsite in the winter and spring, but only for calves. No night shelter was provided in the eastern region. Calves were tethered and kept in the overnight shelter until morning, when the yak cows were milked. During the day, a majority of the herds visited allowed the calves to graze with the cows and they were brought to the night shelter in the evening, while a few (west, 3 herds; central, 1 herd; east, 0 herd) did not allow calves to graze together with cows. In general, cows were gathered from the rangeland and brought to the campsite for milking in the morning. The calves were allowed to suckle for a short time to stimulate the milk let-down and were then tethered next to the cow. Cows were generally either tied by the horns or limbs with a short rope during milking. During the time of the visit, lactating yak cows were milked once a day, usually between 0530 and 0830h. With the exception of one herd in the eastern region, no fixed milking order was followed.

All herders mentioned that they did not assist yak cows during calving. The median daily milk yield per cow was 1,000 g (east, 1,250 g; central, 750 g; west, 2,250 g) in summer and 350 g (east, 500 g; central, 300 g; west, 500 g) in winter. In the eastern yak-farming region, most herders do not milk cows in winter when feed is scarce, in order to maintain the body condition of animals.

Animal health management practices

The majority of the respondents (east, 81%; central, 100%; west, 100%) mentioned that they had experienced yak mortality in the past year (October 2017 to November 2018) (Table 6). The median yak mortality varied between regions (east, 4.5; central, 7; west, 7) ($P < 0.05$; $\xi^2 = 0.13$). Likewise, there were regional differences in yak mortality percentage (east, 7.1%; central, 16.7%; west, 15.1%; $P < 0.05$). Although yak herders do not keep animal health records, most of the respondents in the central and western regions identified wild predators as the greatest cause of mortality in all animal age groups (Table 6). Most of the respondents in the western region mentioned that gid infestations had contributed to high mortality amongst young animals (< 3 years of age), even though most of these herders had dewormed their young animals (Table 6). The finding that 64% of the herders (western region) reported this problem indicates the seriousness of this issue, and there is a need to communicate more about gid disease to these rural communities (including non-yak farming villages). Some herders (east, 1; central, 16; west, 2) also reported that they had not dewormed their yaks, because the animals had not exhibited any signs of sickness or diarrhoea. In addition, the majority of the respondents

Table 6 Percentage of herders per answer for four health variables and painful practices for three yak-farming regions.

Variables	Eastern (n = 16)	Central (n = 19)	Western (n = 17)
<i>Cause of mortality*</i>			
Predator	23.1	84.2	88.2
Disease (including diarrhoea)	53.9	31.6	5.9
Gid infection	0	0	64.7
Accident	0	15.8	47.1
Other (unknown plant and water poison)	31.3	42.1	0
<i>Frequency of livestock extension services received</i>			
Never	18.7	36.8	41.2
Whenever required	75	63.2	52.9
Sometimes	6.2	0	5.9
<i>Frequency of deworming</i>			
Not at all	6.3	84.2	11.8
Once a month	0	5.3	35.3
Once every 2–3 months	0	0	35.3
Whenever received anthelmintic drug	0	5.3	11.7
When animals are less active, have loss of appetite, diarrhoea	93.7	5.3	5.9
<i>Frequency of ectoparasite treatment</i>			
Not at all	43.7	94.7	88.2
Once every six months	0	0	5.9
When animal rubs or herder observes lice and ticks on yak hair	56.3	5.3	5.9
<i>Painful practices in a herd</i>			
Nose ringing	18.7	0	88.2
Ear tagging	12.5	68.4	0
Dehorning	0	0	6.30
Bull castration	93.7	100	94.1
<i>Bull castration age (years)</i>			
No	6.3	0	5.9
2– ≤ 3	68.7	15.8	0
3– ≤ 4	18.7	63.2	11.8
4– ≤ 5	6.2	5.3	64.7
5– ≤ 6	0	10.5	17.6
Don't know	0	5.3	0

Multiple answers for cause of yak mortality means totals do not add up to 100%.

*Yak mortality cause based on herders who experienced yak mortality.

(central and western regions) said that they had not treated their animals against ectoparasites (Table 6).

More than half of the respondents stated that they had sought livestock extension services whenever they were required (Table 6). The distance between the yak herds and the extension service centres probably explains why only a few respondents vaccinated their yaks (east, 1 herd; central, 1 herd; west, 0 herd). Moreover, most respondents in the central (84%) and western (100%) regions also did not use indigenous medicines to treat sick animals. About 75% of the respondents in the eastern region reported that they fed aconite (*Aconitum* spp) root extract to sick animals to treat for parasitic infections and inflammatory conditions.

Painful management practices

All of the respondents mentioned that either they or their neighbouring herders had performed painful procedures on yaks. A few young female animals had received ear tags to enhance the attractiveness of the herd and make animal identification easier. In this procedure, the ear of an animal is pierced with a stitching pin and the tag inserted. The ear tags (which should not be confused with common ear tags used for cattle and pigs) are made of short bunches of brightly coloured yak-tail hair. In the herds that were visited, one to three calves per herd in the eastern (four herds) and central (two herds) regions had ear tags. Likewise, between one and four cows in each of three herds (east) and 1–12 cows in each of eight herds (central) had ear tags. A few respondents in the eastern region indicated that they weaned older calves (about two years of age) by inserting a temporary nose ring or stick (a piece of bamboo, sharpened at the end, piercing the nasal septum, and then a piece of leather is stitched to the bamboo serving as a nose flap) when the dam calved again in the following year, and the older calf still attempted to suckle. Although the gestation period of a yak cow (250–260 days) is similar to that of a dairy cow, calving occurs every 2–3 years (Zi 2003) which is probably due to reduced health conditions as a result of poor fluctuations in availability of good nutrition, which also explains the relatively late weaning of calves. Most of the respondents in the western region reported inserting a wooden stick through the nasal septum of bulls used for transportation and ploughing to be common.

In the herds that were visited, most respondents reported that male yaks not used for breeding were castrated between the age of two and six years. In most cases, the herders incised the scrotum at the lower part, after which they pulled the testicles, either cutting the spermatic cords with a knife or pulling them until they broke. In one herd in the central region, a Burdizzo device was used. The Burdizzo is a castration clamp intended to crush the blood vessels, which leads to loss of blood supply to the testes. The advantage of using a Burdizzo device to castrate bulls is that it seems to cause less acute pain compared to surgical or rubber-ringed castration (Stafford & Mellor 2005). Five herders (east, 0; central, 4; west, 1) used iodine as an antiseptic after castration. Although all of the respondents believed animals to have feelings and the potential to experience

stress, they did not use anaesthesia or analgesia during or after painful procedures.

All respondents reported naming animals in order to aid identification (east, 31.8%; central, 19.1%; west, 40.0%), to facilitate gathering them (east, 63.6%; central, 76.2%; west, 56.0%) and because of the traditional practice of name-giving (east, 4.6%; central, 4.7%; west, 4.0%).

Avoidance distance

The avoidance distance of calves and lactating cows to an unknown person is displayed in Figure 2. The avoidance distance for calves differed significantly between regions (LMM, $F = 35.68$, $df = 2$; $P < 0.001$). The avoidance distance of calves in the central and western region was shorter than that of calves in the eastern region ($P < 0.001$). No regional differences were observed in the avoidance distance of lactating cows (LMM, $F = 2.28$, $df = 2$; $P = 0.10$).

The mean percentage of calves that could be touched by an unfamiliar observer was higher in the western region (41%, range: 0–71%) than in eastern (6%, range: 0–14%) and central (12%, range: 0–50%) regions (GLMM, $F = 17.14$, $df = 2$; $P < 0.001$). The mean percentage of lactating cows that could be touched by an unfamiliar observer was higher in the western (16%, range: 0–55%) and eastern (11%, range: 0–75%) regions than it was centrally (5%, range: 0–50%) (GLMM, $F = 3.24$, $df = 2$; $P = 0.02$).

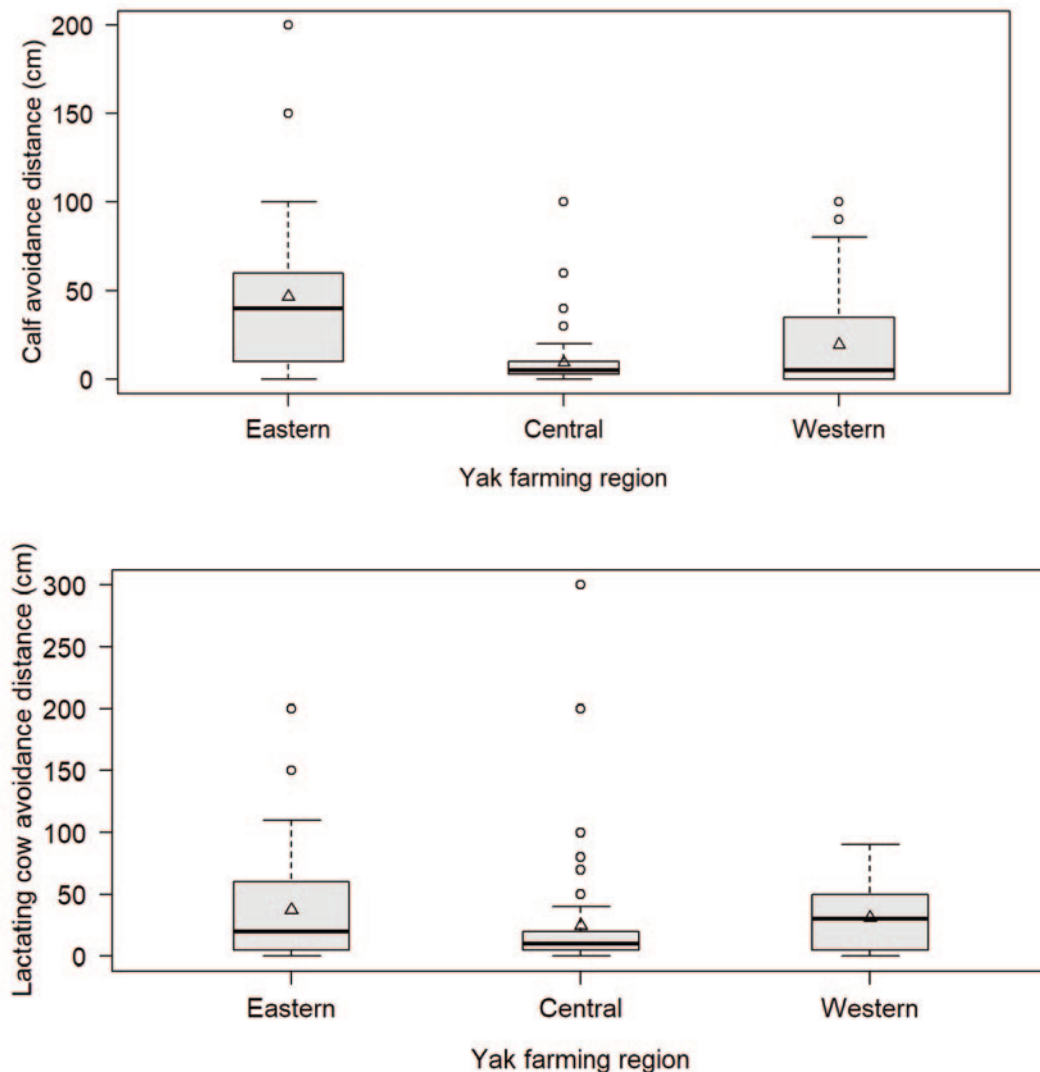
Herd health

Six health parameters were assessed in lactating cows and calves during the milking procedure (Table S7). Of the 567 cows and 549 calves assessed, six cows (east, 0.9%; central, 3.2%; west, 1.4%) and five calves (east, 1.1%; central, 1.3%; west, 3.3%) were found to have lesions. Three cows (east, 0.9%; central, 0.0%; west, 1.3%) and three calves (east, 0.0%; central, 1.3%; west, 2.0%) were lame. No evidence of nasal discharge was observed in any of the herds that were visited. Ocular discharge was observed in four cows (east, 1.7%; central, 3.2%; west, 3.3%) and three calves (east, 3.3%; central, 0.0%; west, 0.0%). No signs of diarrhoea were observed in any of the herds. Nine cows (east, 5.2%; central, 3.2%; west, 0.7%) and eleven calves (east, 1.1%; central, 10.0%; west, 2.0%) were assessed as being dirty on one side of the body and/or on a hind limb. In each herd, one to two calves were assessed as being dirty on one side of the body (east, 1; central, 1; west, 3) and/or hind limb (east, 1; central, 4; west, 2). Similarly, one to two cows per herd were assessed as being dirty on one side of the body (east, 2; central, 1; west, 2) and/or hind limb (east, 1; central, 4; west, 2). At the time of the visits, none of the animals in the herds were observed as having had nose rings or sticks inserted.

General herd behaviour

The percentage of animals eating, lying down, standing idle, walking and exhibiting other behaviours (suckling, licking) is presented in Figure 3. In the morning, there were no regional differences in the number of yaks eating (LMM, $F = 1.04$, $df = 2$; $P = 0.35$), lying down (LMM, $F = 2.40$, $df = 2$; $P = 0.09$), standing idle (LMM, $F = 0.24$,

Figure 2



The avoidance distance (cm) for calves (top) and lactating cows (bottom) to an unknown person in three yak-farming regions. The boxplot shows the median (horizontal line), mean (triangle), and 25th and 75th percentile. The whiskers extend to the 10th and 90th percentile. Means of bars with different letters within each region are significantly different ($P < 0.05$).

$df = 2$; $P = 0.79$) or exhibiting other behaviours (LMM, $F = 0.04$, $df = 2$; $P = 0.96$). A regional difference was found in walking behaviour (LMM, $F = 5.51$, $df = 2$; $P < 0.05$), and it is likely yaks of the central and western regions need to be walked more in search for forage in winter and spring (when the herds were visited). Similarly, in the afternoon, there were no regional differences in the percentage of yaks eating (LMM, $F = 2.844$, $df = 1$; $P = 0.19$), lying down (LMM, $F = 2.56$, $df = 1$; $P = 0.20$), standing idle (LMM, $F = 5.430$, $df = 1$; $P = 0.08$), walking (LMM, $F = 2.07$, $df = 1$; $P = 0.26$) or exhibiting other behaviours (LMM, $F = 0.248$, $df = 1$; $P = 0.66$).

Self-grooming and social behaviour

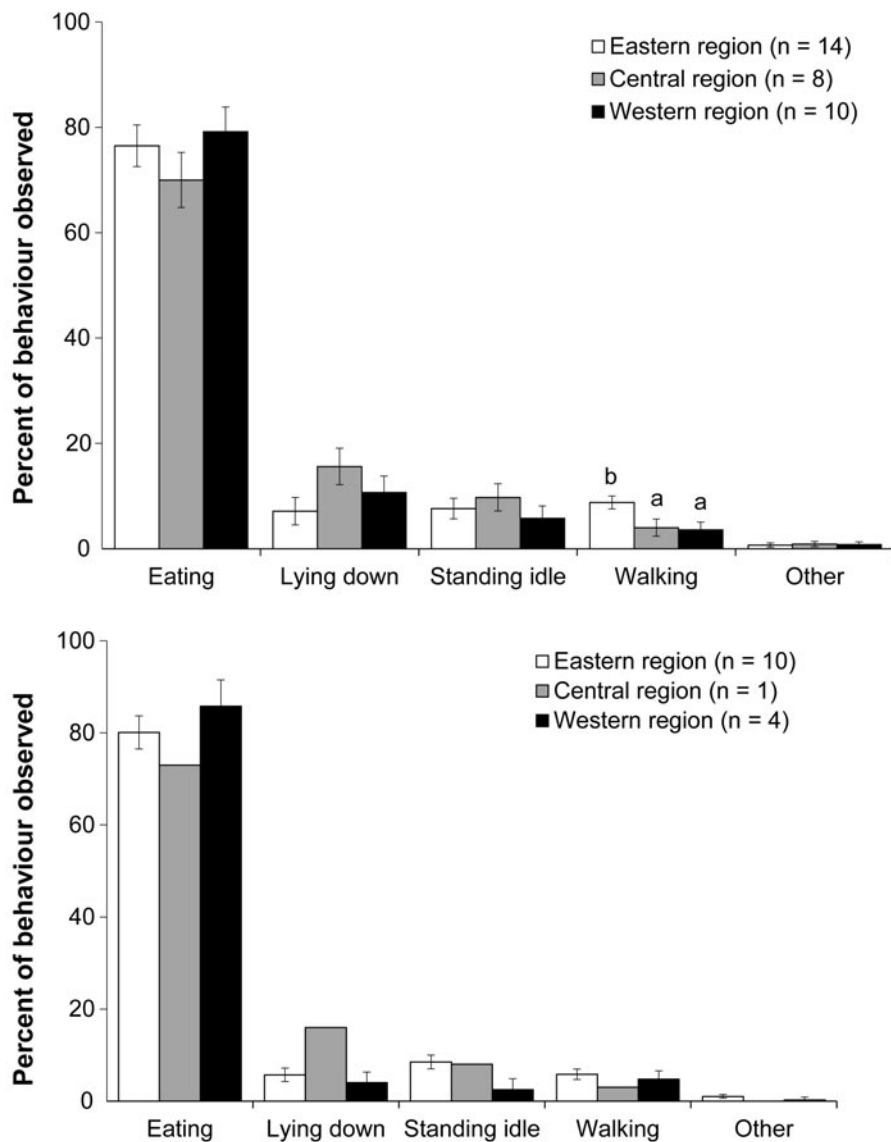
A total of 3,549 behavioural events were counted, with the majority of these consisting of self-grooming (self-licking, 62%; self-scratching/rubbing, 22%), followed by agonistic

behaviour (6%), flehming behaviour (5%), allogrooming (2%), and playing/other behaviour (3%). In the morning, central-region yaks exhibited more self-licking (0.83 times per animal in 15 min) than western (0.43 times per animal in 15 min) or eastern yaks (0.29 times per animal per 15 min) (Table 8). Furthermore, yaks in the central and western regions tended to exhibit more scratching and rubbing than those in the eastern region (Table 8). However, yaks in the eastern region tended to exhibit slightly more head butting and chasing than those in the other two regions. It is important to note that only one observation was available for the central region, and it was therefore excluded from the analysis (Table 8).

Discussion

This study assessed the welfare of free-ranging yaks, and especially lactating cows and suckling calves, under field

Figure 3



Mean (\pm SEM) yak behavioural states observed in the morning (0625–1200h; top) and afternoon (1500–1750h; bottom) when on the rangeland in three yak-farming regions. Means of bars with different superscripts within each behaviour differ significantly ($P < 0.05$).

conditions. The fact that data were collected under field conditions has both advantages and disadvantages. On the one hand, herds were studied while performing their natural behaviour in their natural surroundings, providing information about their behavioural states and interactions. While, on the other, the field conditions made it hard to approach animals closely which, in combination with the agitated temperament of yaks, meant we were unable to examine the health conditions of the animals at close range. This implies that certain welfare issues may have been overlooked.

Animal health and management

Most of the yaks observed were clean, indicating that they had access to clean, dry areas in which to lie down, although no bedding was provided in the rangeland. Integumentary

alterations were virtually absent. It is important to note, however, that the observations were performed at a distance (3–6 m between the observer and the animals) in order to avoid stressing the animals, and most parts of the animals' bodies were covered with long hair, which impaired visual detection. This result might therefore be an underestimation. The low prevalence of lame yaks implies that the management system and living conditions created a low risk of leg and hoof problems. Our findings further support the benefits of animals having free access to rangeland with respect to cleanliness, integumentary injuries and locomotion (Popescu *et al* 2013; Zuliani *et al* 2018).

The low number of cases of ocular discharge and the absence of nasal discharge indicate that few, if any, infections of the eyes and respiratory system were present in the herds that were visited. Although no yaks were observed to

Table 8 Mean (min–max) number of events per animal per 15 min per type of behaviour for three yak-farming regions in Bhutan.

Activity	East	Central	West
<i>Head butting</i>			
Morning (n = 32)	0.016 (0–0.050)	0.003 (0–0.010)	0.007 (0–0.0300)
Evening (n = 16)	0.017 (0–0.065)	0	0.004 (0–0.018)
<i>Chasing</i>			
Morning (n = 32)	0.041 (0–0.130)	0.021 (0–0.040)	0.017 (0–0.060)
Evening (n = 16)	0.039 (0–0.134)	0	0.025 (0–0.046)
<i>Fighting</i>			
Morning (n = 32)	0.009 (0–0.060)	0.014 (0–0.050)	0.008 (0–0.040)
Evening (n = 16)	0.017 (0–0.099)	0	0.003 (0–0.016)
<i>Adult-young licking</i>			
Morning (n = 32)	0.036 (0–0.180)	0.148 (0–0.460)	0.095 (0–0.270)
Evening (n = 16)	0.059 (0–0.217)	0.106	0.182 (0.042–0.541)
<i>Adult-adult licking</i>			
Morning (n = 32)	0.004 (0–0.30)	0.018 (0–0.050)	0.025 (0–0.100)
Evening (n = 16)	0.003 (0–0.017)	0	0
<i>Self-licking</i>			
Morning (n = 32)	0.294 (0.100–0.590)	0.826 (0.570–1.540)	0.426 (0.110–0.930)
Evening (n = 16)	0.273 (0.106–0.513)	0.228	0.417 (0.160–0.976)
<i>Scratching and rubbing</i>			
Morning (n = 32)	0.106 (0.040–0.230)	0.199 (0.100–0.340)	0.203 (0.020–0.340)
Evening (n = 16)	0.123 (0.014–0.326)	0.083	0.179 (0.073–0.290)
<i>Flehming behaviour</i>			
Morning (n = 32)	0.080 (0.020–0.400)	0.032 (0–0.090)	0.050 (0–0.120)
Evening (n = 16)	0.070 (0.012–0.178)	0.032	0.083 (0.014–0.159)
<i>Play behaviour</i>			
Morning (n = 32)	0.016 (0–0.040)	0.005 (0–0.030)	0.011 (0–0.020)
Evening (n = 16)	0.039 (0–0.158)	0.011	0.013 (0–0.032)

have diarrhoea, some of the herders responded that there had been a few cases of diarrhoea. These animals might have been missed by the scoring method applied, or they might have been out of sight during the observations. Herders in the eastern and central regions further acknowledged diarrhoea to be common amongst yaks in the spring and summer, possibly leading to mortality. This is probably due to changes in roughage intake from dry roughage with poor nutritive value to fresh, green roughage with high nutritive value (Zuliani *et al* 2018). Worm infestations (eg *Ascaris* spp) are another potential cause of diarrhoea. A previous study reported 51% of 118 yak faecal samples to

have endoparasites present (Tshering 2015). Most herders indicated that they do not deworm their animals, or that they deworm them only if they are exhibiting signs of diarrhoea. Parasitic infections can cause malnutrition and reproduction disorders in animals (Sevá *et al* 2018). In addition, most of the sources of drinking water in the central yak-farming region were classified as dirty and may have been contaminated by wild boars and other wild animals. Some yak herds (central) were grazing together with cattle in the winter rangeland, and these yaks will have been at a greater risk of contracting diseases, such as foot and mouth disease, bovine viral diarrhoea virus (Mishra *et al* 2008) and

Cryptosporidium spp (Qin et al 2014). All these potential causes of infection could contribute to morbidity and mortality among yaks.

Another factor herders identified as playing a major role in wounds, lesions and mortality were predators — including tigers (*Panthera tigris*), Asian black bears (*Ursus thibetanus*), snow leopards (*Panthera uncia*) and Asiatic wild dogs (*Cuon alpinus*). Herders mentioned that cows and their calves were gathered close to the campsite in the evening only during the lactation period, and that they survey the rest of their animals every three to seven days. This lack of supervision could explain why wild predators are afforded the opportunity to catch yak as prey.

Three painful management practices were identified: piercing of the nasal septum, castration and ear tagging. Piercing of the nasal septum is, as indicated by the herder, sometimes carried out on calves due to be weaned. However, at the time of visit no calves were observed to have a stick through the nose, which was probably because the visits were not conducted during the period calves are normally weaned. Another reason to pierce the nasal septum is to insert a nose ring. This is only applied to castrated bulls during ploughing and transporting. Applying tension to the rope attached to the nose ring to enable the animal to be guided and controlled, inflicts pain as the tissue of the nasal septum is highly sensitive to pressure (Alam et al 2010).

The traditional method of castration is an important welfare concern, as it is carried out without analgesia. Moreover, bulls are rarely handled by herders which makes it highly likely that being restrained and held to the ground for castration causes the bulls a high degree of stress. Both the forelimb and hindlimb of an animal are tied into a figure-of-eight with a long, double-looped rope and the herder applies force from the end of a rope until the forelimb touches the hindlimb of the animal or *vice versa*, and the animal falls to the ground. In Bhutan, bulls are castrated to avoid unwanted breeding in a herd with castrated bulls usually sold to be slaughtered, and only a few strong individuals trained as working animals. However, slaughter of adult yaks has declined in the past 15 years because of increasing religious sentiments among yak herders in Bhutan (Dorji et al 2020). Herders castrate bulls when they are mature instead of when they are young since it is easier to palpate the scrotum during the castration procedure. Wounds resulting from castration heal more slowly in mature bulls, perhaps prolonging the pain (Norring et al 2017).

Finally, ear tagging of young female yaks was mentioned as a painful management practice. This practice is largely comparable with putting modern ear tags in the ears of cattle, pigs or sheep, despite not being applied using modern-day techniques. Increased awareness amongst herders concerning the welfare impact of piercing the nasal septum, castration and ear tagging, and improving their surgical procedures could reduce this animal welfare impairment. It could also be reduced through training provided by livestock professionals or local livestock extensionists, as well as by improved access to health services.

Avoidance distance and management

Central region yaks exhibited smaller avoidance distances to an unknown human than those from the other two regions. In general, avoidance distance reflects the quality and frequency of interactions between a human and the animals (Waiblinger et al 2006; Battini et al 2011). Unlike in the eastern and western regions, the winter rangeland in the central region is linked to a main road. It could be that the yaks in the central region were more accustomed to novel stimuli (eg unfamiliar humans and approaching vehicles). The amount and type of interactions that the herders had with their animals could be another factor perhaps affecting responses of yaks to an unfamiliar human. It was perceived, without registering human-animal interactions, that female herders were more empathetic than their male counterparts which is in accordance with other studies (Herzog et al 1991; Lensink et al 2000; Kiliç & Bozkurt 2013). In the herds that were visited, more than half of the herders were female, especially in the central (73%) and western (82%) regions. This could also be an explanatory factor why the yaks in these two regions exhibited a smaller avoidance distance to an unfamiliar human than those in the eastern region. The interviews also revealed that herders in all of the yak-farming regions were 18 years of age or older. Previously, children would accompany their parents, assisting in herding yaks and attaining the skills required to properly care for their animals. Nowadays, children are sent away to school, returning to the village only after completing their education (if, indeed, they return at all). Literate young pastoralists are less interested in taking up yak farming, which they tend to view as an old-fashioned, traditional means of earning a livelihood (Wangchuk & Wangdi 2015; Dorji et al 2020). Access to education and alternative sources of income are likely to increase the workload for herders (parents), possibly reducing the amount of time that they are able to spend on their animals, thus resulting in a less intimate human-animal relationship (Lensink et al 2000).

Other factors that might have had an effect on the avoidance-distance measurements include the feedings before milking and the ways in which the animals were collected for milking. All of the herders hand-fed maize flour mixed with common salt (east) or wheat flour mixed with turnips or other forage (central and west) to calves and lactating cows in order to achieve quick co-operation. Although the avoidance-distance test for the yak cows was performed after milking, our results may have been affected by this practice (Windschnurer et al 2008; Ebinghaus et al 2016). In addition, all of the herders in the survey reported that their yak cows had names and that they were called by their names when they were gathered for milking. Studies have shown that calling cows by name and talking to them softly during milking can neutralise fear of humans or other negative animal emotions (Bertenshaw & Rowlinson 2009; Lürzel et al 2018). All of these practices affect the ways in which animals react to humans.

Yak free-range behaviour

We observed a relatively high percentage of self-grooming in the yak herds in the eastern region. The fact that none of the yaks that we observed were dirty (or very dirty) might indicate a high ectoparasite load (Mooring & Samuel 1998). This is supported by the observed scratching and rubbing (not necessarily in the same herds as some yak herds were allowed to graze together), as ectoparasites cause skin irritation, thus leading animals scratch and rub their body parts often. Unfortunately, the presence of ectoparasites was not evaluated. In an earlier study, the presence of ectoparasites (eg lice, fleas, ticks) in yaks were reported in the eastern yak-farming region of Bhutan (Tshering 2015). Moreover, the yaks in the central and western regions are rarely treated against ectoparasites, which might explain these observations.

Yaks exhibited a high level of synchronicity of eating during the time of our visit. The percentage of yaks eating (34–98%) was close to the reported 34 to 80% in free-ranging Chinese yaks (Wiener *et al* 2003), but wider than the 42–64% range observed in wild yaks (Buzzard *et al* 2014). In our study, the difference between herds in terms of time spent eating might have been affected by time of day, weather condition, the quality and availability of forage in the rangeland, season, and the age and sex of the animals. For example, the quality and quantity of forage in the rangeland was apparently lower in the central region than in the other two regions, probably because the rangelands were also used by cattle. The poor quality and quantity of forage in the rangeland might explain why the yaks in the central region spent slightly less time eating (and more time standing idle or ruminating) than those in the eastern and western regions (Arave & Albright 1981; Luming *et al* 2008). In the winter and spring, when forage is scarce, yaks must cover large distances in search of forage in order to meet their daily energy requirements. When weather conditions are less favourable, yaks spend reduced searching for forage, in order to limit energy expenditure (Liu *et al* 2019). This reflects the manner in which yaks adapt their grazing strategies to weather conditions and the availability of forage.

The visits to the yak herds in the eastern region in October coincided with the breeding season, which is from June to November (Wiener *et al* 2003). The presence of a few yak cows in oestrus during the time of our visit in the eastern region may account for the slightly higher counts of agonistic and flehming behaviour, as compared to the central and the western regions. Nevertheless, the low percentage of agonistic behaviour observed and the presence of only a few integumentary lesions in the yaks that were observed suggests social stability within the herds or that the large amount of space available to the yaks allows them to avoid conflicts. In addition to maintaining the cleanliness of their own bodies, animals perform social licking in order to reduce tension (Sato *et al* 1991).

In this study, the welfare of yaks was evaluated once. Since the yaks just returned from the summer rangelands where good quality feed is available in sufficient quantities, the welfare status of the animals is probably at its best during this

period. Nevertheless, our findings can still serve as a baseline for further studies (eg on the ways in which behaviours are driven by the hierarchy within the herd). It is important to note, however, that yaks should also be assessed for welfare during other periods of the year, for example, after the winter period when forage may have been limited (Hernandez *et al* 2018). In addition, some of our results might have been influenced by differences in the time of data collection. For example, the yaks in the eastern region exhibited slightly more aggressive and flehming behaviour than those in the central and western regions. In December, the weather conditions tend to be colder and harsher than they are in October, and this is likely to influence the behavioural activities of animals (eg yaks spend less time eating during forage shortages and under harsh weather conditions: Luming *et al* 2008). In future studies, herders or livestock extensionists could potentially be trained to perform the protocol in order to collect welfare and management data all year round.

Animal welfare implications and conclusion

This study is the first to provide an impression of the welfare status of free-ranging yaks kept in open fields under mountainous conditions. In general, it can be concluded that the welfare conditions of the yaks in Bhutan upon returning from the summer rangelands seem to be good based on the applied welfare assessment protocol and that there are only marginal regional differences in yak welfare. However, there are causes for concern that require further investigation. Currently, mortality rates are high, and animals are mutilated and castrated without the use of pain medication. Also, the water sources available were often scored either partly dirty or dirty, which can be potentially harmful to animals, impairing their welfare. The high levels of rubbing and scratching behaviour, together with large numbers of gid infestations (as reported by herders) may indicate parasites to be a serious welfare concern in yaks. Combined with reduced access to veterinary or extension services, this should be evaluated in more detail in further studies on yak health and welfare. Finally, the welfare status of these yaks might differ in other seasons of the year when they are potentially exposed to harsh weather conditions and limited feed availability.

Declaration of interest

None.

Acknowledgements

This project (PhD) was funded by The Netherlands University Foundation for International Cooperation (NUFFIC). We thank the livestock extensionists and local health workers of the study sites for giving their support to this study. The authors also thank the yak herders for participating in the study and Linda Karr for proof-reading the manuscript.

References

- Alam MR, Gregory NG, Uddin MS, Jabbar MA, Chowdhury S, Sharmin and Debnath NC 2010 Frequency of nose and tail injuries in cattle and water buffalo at livestock markets in Bangladesh. *Animal Welfare* 19: 295-300

- Appleby MC and Mitchell LA** 2018 Understanding human and other animal behaviour: Ethology, welfare and food policy. *Applied Animal Behaviour Science* 205: 126-131. <https://doi.org/10.1016/j.applanim.2018.05.032>
- Arave CW and Albright JL** 1981 Cattle behavior. *Journal of Dairy Science* 64: 1318-1329
- Bates D, Mächler M, Bolker BM and Walker SC** 2015 *lme4: Linear Mixed-effects Models Using Eigen and S4 R Package Version 1*. <https://CRAN.R-project.org/package=lme4>
- Battini M, Andreoli E, Barbieri S and Mattiello S** 2011 Long-term stability of avoidance distance tests for on-farm assessment of dairy cow relationship to humans in alpine traditional husbandry systems. *Applied Animal Behaviour Science* 135: 267-270. <https://doi.org/10.1016/j.applanim.2011.10.013>
- Bertenshaw C and Rowlinson P** 2009 Exploring stock managers' perceptions of the human-animal relationship on dairy farms and an association with milk production. *Anthrozoös* 22: 59-69. <https://doi.org/10.2752/175303708X390473>
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH and White JSS** 2009 Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* 24: 127-135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Buzzard PJ, Xu D and Li H** 2014 Sexual/aggressive behavior of wild yak (*Bos mutus* Prejevalsky 1883) during the rut: influence of female choice. *Chinese Science Bulletin* 59: 2756-2763. <https://doi.org/10.1007/s11434-014-0247-0>
- Derville M and Bonnemaire J** 2010 Marginalisation of yak herders in Bhutan: can public policy generate new stabilities that can support the transformation of their skills and organisations? and bonds to territories: a case study in France and Brazil. In: Coudel E, Devautour H, Soulard C and Hubert B (eds) *Proceedings of the ISDA 2010* pp 10. Cirad-Inra-SupAgro: Montpellier, France
- Dhendup R** 2015 *Phenotypic characterization of yak hybrids (Dzo and Dzom) at Sakteng gewog*. Department of Animal Science pp 68. College of Natural Resources, Royal University of Bhutan: Lobesa, Punakha, Bhutan
- des Roches AB, Veissier I, Boivin X, Gilot-Fromont E and Mounier L** 2016 A prospective exploration of farm, farmer, and animal characteristics in human-animal relationships: An epidemiological survey. *Journal of Dairy Science* 99: 5573-5585. <https://doi.org/10.3168/jds.2015-10633>
- Dinno A** 2017 *Dunn's test of multiple comparisons using rank sums*. <https://cran.r-project.org/web/packages/dunn.test/dunn.test.pdf>
- DoL** 2018 *Livestock Statistics 2018* pp 342. Ministry of Agriculture and Forests: Thimphu: Bhutan
- Dorji N, Derks M, Dorji P, Groot Koerkamp PWG and Bokkers EAM** 2020 Herders and livestock professionals' experiences and perceptions on developments and challenges in yak farming in Bhutan. *Animal Production Science* 60: 2004-2020. <https://doi.org/10.1071/ANI9090>
- Ebinghaus A, Ivmeyer S, Rupp J and Knierim U** 2016 Identification and development of measures suitable as potential breeding traits regarding dairy cows' reactivity towards humans. *Applied Animal Behaviour Science* 185: 30-38. <https://doi.org/10.1016/j.applanim.2016.09.010>
- Edmonson AJ, Lean IJ, Weaver LD, Farver T and Webster G** 1989 A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science* 72: 68-78
- Flower FC and Weary DM** 2006 Effect of hoof pathologies on subjective assessments of dairy cow gait. *Journal of Dairy Science* 89: 139-146. [https://doi.org/10.3168/jds.S0022-0302\(06\)72077-X](https://doi.org/10.3168/jds.S0022-0302(06)72077-X)
- Fox J and Weisberg S** 2019 *An R Companion to Applied Regression, Third Edition*. Sage Publications: CA, USA
- Gyamtsho P** 2000 Economy of yak herders. *Journal of Bhutan Studies* 2: 1-45
- Hernandez A, Berg C, Westin R and Galina C** 2018 Seasonal differences in animal welfare assessment of family farming dual-purpose cattle raised under tropical conditions. *Animals* 8: 125. <https://doi.org/10.3390/ani8070125>
- Herzog HA, Betchart NS and Pittman RB** 1991 Gender, sex role orientation, and attitudes toward animals. *Anthrozoös* 4: 184-191
- Kiliç I and Bozkurt Z** 2013 The relationship between farmers' perceptions and animal welfare standards in sheep farms. *Asian-Australasian Journal of Animal Sciences* 26: 1329-1338
- Lensink J, Boissy A and Veissier I** 2000 The relationship between farmers' attitude and behaviour towards calves, and productivity of veal units. *Annales Zootechnie* 49: 313-327
- Lenth RV** 2016 Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software* 69: 1-33
- Liu P, Ding L, Zhou Y, Jing X and Degen AA** 2019 Behavioural characteristics of yaks grazing summer and winter pastures on the Qinghai-Tibetan Plateau. *Applied Animal Behaviour Science* 218: 1048262. <https://doi.org/10.1016/j.applanim.2019.06.007>
- Luming D, Ruijun L, Zhanhuan S, Changting W, Yuhai Y and Songhe X** 2008 Feeding behaviour of yaks on spring, transitional, summer and winter pasture in the alpine region of the Qinghai-Tibetan plateau. *Applied Animal Behaviour Science* 111: 373-390. <https://doi.org/10.1016/j.applanim.2007.06.008>
- Lürzel S, Barth K, Windschnurer I, Futschik A and Waiblinger S** 2018 The influence of gentle interactions with an experimenter during milking on dairy cows' avoidance distance and milk yield, flow and composition. *Animal* 12: 340-349. <https://doi.org/10.1017/S1751731117001495>
- Mishra N, Vilcek S, Rajukumar K, Dubey R, Tiwari A, Galav V and Pradhan HK** 2008 Identification of bovine viral diarrhoea virus type 1 in yaks (*Bos poeaphagus grunniens*) in the Himalayan region. *Research in Veterinary Science* 84: 507-510. <https://doi.org/10.1016/j.rvsc.2007.05.019>
- Mooring M and Samuel W** 1998 Tick defense strategies in Bison: the role of grooming and hair coat. *Behaviour* 135(6): 693-718. <https://doi.org/10.1163/156853998792640413>
- Norring M, Mintline EM and Tucker CB** 2017 The age of surgical castration affects the healing process in beef calves. *Translational Animal Science* 1: 358-366. <https://doi.org/10.2527/tas2017.0044>
- Popescu S, Borda C, Diugan EA, Spinu M, Groza IS and Sandru CD** 2013 Dairy cows welfare quality in tie-stall housing system with or without access to exercise. *Acta Veterinaria Scandinavica* 55: 43-43
- Qin S, Zhang X, Zhao G, Zhou D, Yin M, Zhao Q and Zhu X** 2014 First report of *Cryptosporidium* spp in white yaks in China. *Parasites & Vectors* 7: 230
- R Team Core** 2018 *R: A language and environment for statistical computing*. R Foundation for Statistical Computing: Vienna, Austria. <http://www.R-project.org/>

- Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D and Ripley MB** 2013 Package 'MASS', *Cran R*. <http://www2.uaem.mx/r-mirror/web/packages/MASS/MASS.pdf>
- Sargeant JM, Leslie KE, Shirley JE, Pulkrabek BJ and Lim GH** 2001 Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. *Journal of Dairy Science* 84: 2018-2024. [https://doi.org/10.3168/jds.S0022-0302\(01\)74645-0](https://doi.org/10.3168/jds.S0022-0302(01)74645-0)
- Sato S, Sako S and Maeda A** 1991 Social licking patterns in cattle (*Bos taurus*): influence of environmental and social factors. *Applied Animal Behaviour Science* 32: 3-12
- Sevá AD, Pena HF, Nava A, Sousa AO, Holsback L and Soares RM** 2018 Endoparasites in domestic animals surrounding an Atlantic Forest remnant, in São Paulo State, Brazil. *Brazilian Journal of Veterinary Parasitology* 27(1): 13-19. <http://dx.doi.org/10.1590/S1984-29612017078>.
- Stafford KJ and Mellor DJ** 2005 The welfare significance of the castration of cattle: A review. *New Zealand Veterinary Journal* 53(5): 271-278
- Tshering P** 2015 Seasonal prevalence of parasites in yak (*Bos grunniens*) in Sakteng geog. Department of Animal Science pp 50. College of Natural Resources, Royal University of Bhutan: Lobesa, Punakha, Bhutan
- Waiblinger S, Boivin X, Pedersen V, Tosi MAMJ, Visser EK and Jones RB** 2006 Assessing the human-animal relationship in farmed species: A critical review. *Applied Animal Behaviour Science* 101: 185-242. <https://doi.org/10.1016/j.applanim.2006.02.001>
- Wangchuk K and Wangdi J** 2015 Mountain pastoralism in transition: Consequences of legalizing Cordyceps collection on yak farming practices in Bhutan. *Pastoralism* 5: 4. <https://doi.org/10.1186/s13570-015-0025-x>
- Welfare Quality®** 2009 *Welfare Quality® assessment protocol for cattle*. Welfare Quality® Consortium: Lelystad, The Netherlands
- Wiener G, Jianlin H and Ruijun L** 2003 *The Yak, 2nd Edition*. FAO: Bangkok, Thailand
- Windschnurer I, Schmied C, Boivin X and Waiblinger S** 2008 Reliability and inter-test relationship of tests for on-farm assessment of dairy cows' relationship to humans. *Applied Animal Behaviour Science* 114: 37-53. <https://doi.org/10.1016/j.applanim.2008.01.017>
- Zuliani A, Mair M, Kraševc M, Lora I, Brscic M, Cozzi G, Leeb C, Zupan M, Winckler C and Bovolenta S** 2018 A survey of selected animal-based measures of dairy cattle welfare in the Eastern Alps: Toward context-based thresholds. *Journal of Dairy Science* 101: 1428-1436. <https://doi.org/10.3168/jds.2017-13257>
- Zi XD** 2003 Reproduction in female yaks (*Bos grunniens*) and opportunities for improvement. *Theriogenology* 59: 1303-1312. [https://doi.org/10.1016/S0093-691X\(02\)01172-X](https://doi.org/10.1016/S0093-691X(02)01172-X)